The Sum1/Ndt80 Transcriptional Switch and Commitment to Meiosis in *Saccharomyces cerevisiae*

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INTRODUCTION

A key decision in the life of a diploid yeast cell is whether to undergo meiosis and form spores. Sporulation is induced by starvation. Glucose and nitrogen are inhibitors of sporulation, and acetate (a "poor" carbon source) activates the program (65, 77). Commitment to meiosis was first studied using return-to-growth (RTG) protocols in which cells that had been transferred to sporulation medium (acetate) were transferred back to rich growth medium (yeast extract-peptone-dextrose [YPD]) at various times. Rather than continue through meiosis, these cells reentered the mitotic cell cycle. These studies demonstrated that cells must be exposed to the inducing signals for sporulation for a defined interval before completion of the program will take place (35, 43, 62, 154, 161). Cells that had entered the program and completed meiotic DNA replication could efficiently return to growth. Even more surprising, cells that were undergoing meiotic recombination could return to growth. The ability to return to growth declines dramatically around the time that cells exit meiotic prophase and enter the first meiotic division (MI). More recent microscopic studies of live cells showed that cells that had completed MI prior to transfer to rich medium formed spores, while cells that had not yet completed MI exited the meiotic program and resumed vegetative growth (120).

Meiotically induced cells that were transferred to water at time points when rich medium caused RTG efficiently completed meiosis and spore formation (161). These studies led to the concept of "readiness," which occurs prior to meiotic S phase. In addition, a stage at which transfer to rich medium blocked sporulation but did not permit the resumption of mitotic growth (termed partial commitment) was identified. Thus, inhibitory signals (glucose and nitrogen) and activating signals (acetate) differentially control passage through meiotic development, and cells at different stages of meiotic development respond to these signals differently (reviewed in reference 160). These studies led to a model in which cells transit through a series of steps: first "readiness," then "partial commitment," and finally "full commitment". In this article, the point in meiotic development after which cells complete meiosis and form spores even when transferred to rich medium (full commitment) will be referred to as the meiotic commitment point.

The RTG studies described above demonstrate that commitment to meiotic development takes place in prophase. Meiotic prophase has been divided into stages based on the microscopic appearance of chromosomes (199). In leptotene, lateral elements of the synaptonemal complex (SC) are observed. During leptotene, homolog coalignment takes place, the Spo11 endonuclease initiates recombination by introducing double-strand breaks (DSBs) into the genome (81), and these DSBs are further processed into single-stranded nucleoprotein filaments that contain the Rad51 and Dmc1 strand exchange proteins (11, 157). The next

cytological stage of prophase is zygotene, when central regions of the SC, which connect homologs, appear. During this stage, DSBs are processed into either nonrecombinants or joint-molecule (JM) intermediates. Pachytene is defined as the stage when homologous chromosomes are fully connected by continuous tripartite SCs. At this stage, JMs that contain double Holliday junctions have formed but have not yet been resolved, and spindle pole bodies (SPBs) (the yeast equivalent of centrosomes) have duplicated but not yet separated (18). SC disassembly is the cytological feature that defines exit from pachytene. In yeast, pachytene exit is when JMs are resolved as crossovers (4) and when the duplicated SPBs separate to form the MI spindle (18). In organisms with large genomes, SCs can be gradually disassembled during diplotene, and further condensation of chromatids occurs during diakinesis. However, in *Saccharomyces cerevisiae*, SC disassembly is rapid, diplotene is not apparent, and the later changes in condensation are subtle (33). While diplotene can be a significant stage of regulation in other organisms (in mammals, primary oocytes are held in the hormonally regulated diplotene stage [dictyate arrest] for decades), pathways that specifically delay prophase progression after pachytene have not been identified in yeast. As described below, exit from pachytene is the key regulatory transition that regulates progression from meiotic prophase in yeast.

Molecular/genetic studies of meiotic mutants demonstrate that cells blocked at pachytene can efficiently return to growth (159, 196). Ndt80 is the transcription factor that drives exit from pachytene (discussed in more detail below), and the RTG response of the *ndt80Δ* mutant has been well studied (31, 196). In pachytene-arrested *ndt80Δ* cells that have been exposed to rich medium, the SC rapidly disappears and chromatids are segregated in a mitosis-like division (31). While JMs are resolved in the meiotic pathway mainly as crossovers, a distinct pathway that minimizes crossover formation processes JMs during the RTG response, thus maximizing heterozygosity in RTG diploids. Cells in pachytene are therefore able to modify the meiotic recombination pathway in response to nutritional signals to generate outcomes that are beneficial to the vegetative cell. The ability of cells in pachytene to mount a highly regulated RTG response and the inability of cells that have entered the meiotic divisions to return to growth indicate that pachytene exit is closely associated with meiotic commitment.

A temperature-sensitive mutation in *CDK1* (*cdc28-4*), which encodes the cell cycle-regulatory cyclin-dependent kinase (CDK), blocks meiosis in pachytene (159). More recent studies with a mutant form of Cdk1 that is sensitive to cell-permeative ATP analogs (Cdk1-as1) show that exit from pachytene is especially sensitive to Cdk1 inhibition (10). Exit from pachytene is prevented by a checkpoint pathway that is activated in response to persistent recombination intermediates (100). This pathway, termed the recombination checkpoint or pachytene checkpoint (see below), inhibits Cdk1 through the Swe1 protein kinase (which downregulates Cdk1 by phosphorylating a residue near the Cdk1 ATP-binding pocket) (90). These observations indicate that commitment is tightly connected to cyclin-dependent kinase.

While wild-type cells that have entered MI do not normally return to growth, there are certain circumstances in which postmeiotic RTG can take place. This was first described in studies of *SPO14*, which regulates formation of the prospore membrane, a double membranous structure that envelopes haploids following the completion of MII (121, 140). In early studies it was shown
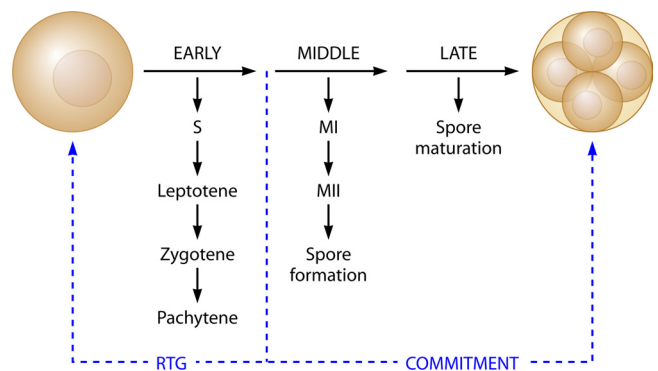

FIG 1 The transcriptional cascade and commitment. The diagram shows a vegetative precursor cell (left) that has been induced by nutrient deprivation to form a spore (right). The early, middle, and late sets of sporulation-specific genes are shown as horizontal arrows, and the meiotic processes that require these gene sets are shown connected by downward arrows. The commitment point is represented as a vertical dashed line, and the alternative responses (return to vegetative growth [RTG] and completion of the sporulation program [commitment]) are indicated as a function of when the inducing signal (starvation) is eliminated (rich medium is added).

that *spo14Δ*-blocked cells could return to growth at postmeiotic stages in meiotic development (62). While this observation led to the suggestion that *SPO14* is a specific regulator of commitment, it was subsequently shown that blocking prospore membrane closure using an *SSP1* (*spo3-1*) mutant or by temperature upshift of wild-type cells also allowed postmeiotic cells to return to growth (63). Notably, the RTG that occurs in postmeiotic *spo14*, *ssp1*, or temperature-upshifted cells takes substantially longer than RTG in precommitment cells. These studies suggest that postmeiotic cells do not normally return to growth because a cellular state has been established in these cells that kinetically favors completion of sporulation and not because mitotic growth has been irreversibly inhibited.

THE TRANSCRIPTIONAL PROGRAM OF MEIOSIS

A key to understanding meiotic regulation is the transcriptional program of meiosis. Genome-wide transcript analyses show that approximately 1,600 yeast genes are differentially expressed during meiotic development (25, 133). About 900 of these genes show similar meiotic expression patterns in distant *Saccharomyces cerevisiae* strain backgrounds (W303 and SK1). This conserved set, which contains about equal numbers of differentially induced and repressed genes, has been termed the core meiotic transcriptome (133). The induced genes can be divided into at least 10 clusters based on their expression patterns. The clusters that are induced shortly after exposure to sporulation medium are enriched in starvation-induced genes. Many of these genes are induced not only in MAT**a**/α strains but also in similarly treated meiosis-deficient (MAT**a**/**a** or MATα/α) strains and are thought to be involved in metabolic and stress responses. Next, clusters of genes, which have been broadly grouped into the early, middle, and late sets, are induced (Fig. 1). The induction of almost all of these genes is MAT**a**/α dependent. Early genes are involved in meiotic DNA replication, homolog pairing, SC formation, and genetic recombination. Middle genes are involved in exit from prophase, the meiotic divisions, and spore formation. Late genes are involved in spore maturation. Approximately 150 genes in the core meiotic transcriptome encode mRNAs that are detectable only in meiotic

cells. These meiosis-specific genes are of particular interest since they superimpose meiotic regulation on the "mitotic default" pathway and control processes that are unique to meiotic development.

Early Genes

Early genes control meiotic induction, S phase, and prophase (Fig. 1). Mechanisms that control early gene expression have been extensively covered in several excellent reviews (77, 113, 179, 180). Below, I present only a broad overview of early promoter regulation and focus on the regulatory relationships that are relevant to meiotic commitment.

Early meiosis-specific promoters are repressed in mitotic cells through Ume6, a DNA-binding protein that recognizes DNA elements termed upstream repression sequences (URS1s) (14, 167). Many early promoters contain URS1s and are derepressed in *ume6Δ* mutants (191). Ume6 also regulates genes that respond to metabolites such as glucose, nitrogen, and inositol (34, 72, 85, 99, 112, 128, 168). Ume6 recruits the Rpd3/Sin3 histone deacetylase complex to URS1s in vegetative cells (75). Full repression of early promoters also requires the Isw2 chromatin-remodeling complex, which also interacts with Ume6 (45).

The induction of early promoters is controlled through Ime1, a transcription factor that has been termed the "master regulator" of entry into meiosis (78, 162). Multiple repression and activation sequences in the unusually long (2.1-kb) *IME1* promoter control its expression (144). It is repressed by the haploid-specific Rme1 DNA-binding protein, and it is also repressed by glucose and by nitrogen (29, 30, 115). Thus, *IME1* is expressed only in starved MAT**a**/MATα diploid cells. In addition, *IME1* expression is activated in response to alkaline media (91, 170) (high pH promotes meiosis) and mitochondrial function (73, 175) (meiosis is restricted to cells that are respiration competent). *IME1* is also repressed by the Cln3 G_1 cyclin (which makes meiotic induction and the mitotic G_1 phase incompatible) (28). The *IME1* promoter therefore functions as a signal integrator of multiple inputs that control entry into sporulation.

Two-hybrid data suggested that Ime1 interacts with Ume6 to form a transcriptional activation complex in meiotic cells, and it has been proposed that a regulated transition from Ume6/Rpd3/Sin3 (repressive) to Ume6/Ime1 (activating) complexes controls early gene expression (16, 138, 189). However, a more recent study indicates that Ume6 is degraded around the time that early genes are induced, in a pathway that requires the Cdc20 E3 ubiquitin ligase and Ime1 (104). This study also showed that a nondegradable form of Ume6 prevents early gene induction. These findings suggest that Ume6 degradation is a prerequisite for early gene expression and are inconsistent with Ume6/Ime1 functioning as a simple transcriptional activation complex. Further work is required to establish how Ime1 is retained at early promoters following Ume6 degradation and to identify the molecular targets of Ime1 that activate transcription.

IME2 was identified in a genetic screen for multicopy plasmids that enabled cells overproducing *RME1* to enter meiosis and undergo meiotic recombination (162). Subsequently, *IME2* overexpression was also shown to permit sporulation in nutrient-rich media (198). *IME2* was the first transcriptional target of Ime1 to be identified, and much of what we know about early meiosis-specific gene regulation was uncovered in molecular/genetic studies of the *IME1/IME2* interaction (113). *IME2* encodes an early
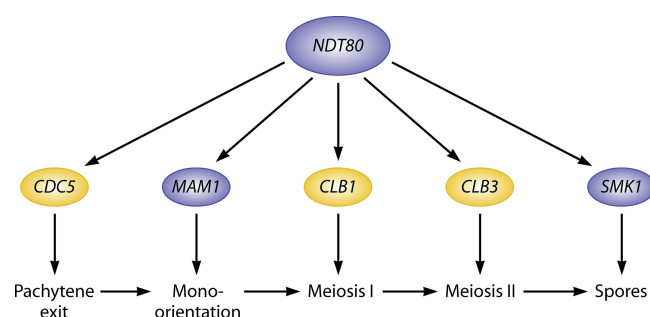

FIG 2 Ndt80 activates multiple processes associated with meiotic commitment and the completion of sporulation. Examples of Ndt80 target genes required for pachytene exit, mono-orientation, MI, MII, and spore formation are shown. The genes shaded in yellow also function in mitotic cells. The genes shaded in blue are repressed by Sum1 in mitotic cells and are meiosis specific.

meiosis-specific CDK-like kinase that controls multiple steps in meiotic development (61, 71). *IME2* promotes early gene expression in an *IME1*-independent pathway (114). It is essential for meiotic DNA replication and the meiotic divisions, and *IME2* regulates the transcriptional program of meiosis at several key steps (Ime2 targets are discussed below).

Nutrient-regulated pathways that reverse early gene expression. RTG studies show that early gene expression is extinguished when rich medium is added to meiotic cells (37). Multiple pathways connect the Ime1 protein to starvation signals. These include a nutritionally regulated glycogen synthase kinase 3-β homolog named Rim11 that promotes the interaction of Ime1 and Ume6 (15, 102, 103, 139). In addition, the Rim15 protein kinase positively regulates Ime1, and Rim15 is inhibited by glucose via the Ras/cyclic AMP (cAMP) pathway (14, 136, 181). The Cln3 G_1 cyclin, which is inhibited by nutritional signals, (6, 42, 49, 66, 129, 130), also restricts Ime1 nuclear localization (28). Genome-wide transcriptional studies of RTG cells demonstrate that the G_1 cyclin gene *CLN3* is induced shortly after the addition of nutrients to meiotic cells, suggesting that Cln3 plays an especially important role in promoting the RTG response (37).

The Ime2 protein kinase is also tightly regulated by nutrients. Ime2 is inhibited by Gpa2, a subunit of the heterotrimeric guanine nucleotide-binding protein that couples to the Gpr1 glucose sensor (32). Ime2 is also inhibited by the Snf1 AMP-activated protein kinase (64). In addition, Ime2 is degraded in response to glucose in a pathway that requires the SCF/Grr1 E3 ubiquitin ligase (46, 135, 145). The inhibition of Ime2 is therefore likely to be involved in promoting the RTG response and reestablishing a regulatory state that permits resumption of mitotic growth. Collectively, these studies demonstrate that nutritional signals can downregulate early gene expression after meiotic induction has occurred through both Ime1 and Ime2 and that the window when early genes are expressed correlates with the precommitted state.

Middle Genes

The middle genes are induced as cells exit pachytene, carry out the nuclear divisions, and form spores. Middle genes can be divided into a subset that function in both mitosis and meiosis and a subset that function exclusively in meiotic cells (Fig. 2). Middle genes that function in both mitosis and meiosis encode proteins such as the B-type cyclins, which activate the M-phase form of Cdk1 (21, 36), and the cell cycle-regulatory polo-like kinase (PLK)

Cdc5, which controls events associated with mitotic and meiotic chromosome segregation (7, 27, 165). These middle genes are often induced in meiotic cells substantially above the level seen in mitotic cells. Middle genes that function exclusively in meiotic cells superimpose meiotic regulation on the cell cycle machinery. An example of a middle gene with a meiosis-specific function is *MAM1*, which controls attachment of chromosomes to the meiotic spindle and promotes the meiosis-specific segregation pattern of chromosomes at MI (107). Other middle genes that function exclusively in meiotic cells are required for the ensuing steps of cellularization and spore wall morphogenesis. As discussed below, the Sum1 repressor and Ndt80 activator proteins control middle promoter induction and can determine whether a given middle promoter is expressed in both mitotic and meiotic cells or exclusively during meiotic development.

The Ndt80 transcription factor. Almost all middle genes contain a consensus DNA element in their promoters termed the middle sporulation element (MSE) (52, 125). Ndt80 is a meiosis-specific transcription factor that binds to MSEs (26, 53). The structure of the Ndt80 DNA-binding domain bound to a near-optimal MSE binding site (consensus, 5′-YGNC*AC***AAAA**-3′ [where N is any base, and Y is C or T]) has been solved (87, 88, 116, 117). Ndt80 recognizes the MSE through an Ig fold, a conserved DNA-binding motif found in the human p53, NF-κB, and STAT transcription factors (141). Similar to other Ig fold transcription factors, Ndt80 recognizes a pyrimidine/G dinucleotide step (corresponding to the underlined AC dinucleotide in the sequence above) and also recognizes the unique minor-groove structure of the poly(A/T) tract (in bold). Consistent with the structural studies, changes to the G/C base pair in the pyrimidine/G dinucleotide step dramatically increase the Ndt80/DNA K_d (dissociation constant), while changes to the bases 5′ to the step affect the K_d less significantly (this will be relevant to understanding how Ndt80 and Sum1 recognize different sets of MSEs as discussed below).

ndt80Δ mutants arrest in pachytene and return to growth with near 100% efficiency (196). The expression of *NDT80* in pachytene-arrested cells leads to the rapid and synchronous entry into MI and completion of sporulation (10, 21). Moreover, Ndt80 is a regulated target of the pachytene checkpoint (26, 53, 176). Thus, by all criteria tested, Ndt80 is required for pachytene exit and is a candidate target of commitment control pathways. Recently it has been shown that the ectopic expression of *CDC5* in *ndt80Δ* pachytene-arrested cells promotes SC disassembly and resolution of JMs into crossovers (57, 165). The transcriptional induction of *CDC5* by Ndt80 therefore drives exit from pachytene. Ndt80 also induces the expression of genes that promote the orientation of sister chromatids toward the same pole of the spindle (monoorientation), nuclear segregation at MI and MII, and spore formation. Thus, the *ndt80Δ* cells expressing Cdc5 do not complete the program. The requirement of Ndt80-inducible genes for the multiple meiotic steps up to and including spore formation underscores the role of Ndt80 in establishing commitment (Fig. 2). In the initial report demonstrating that Ndt80 is the MSE-specific transcription factor, the Herskowitz laboratory showed that *NDT80* is not expressed in *ime1Δ* cells (26). They also showed that Ndt80 activates its own promoter in a positive autoregulatory loop. These results raised the possibility that Ime1 positively regulates *NDT80* transcription, which triggers the autoregulatory loop.

The Sum1 repressor. How is the Ndt80 autoregulatory loop

restricted until pachytene exit? One clue came from the analyses of *SMK1*, a meiosis-specific middle gene that controls spore morphogenesis (86, 182). Analysis of the *SMK1* promoter showed that the MSE that controls its induction (MSE^s) not only activates gene expression during meiosis but also represses gene expression in vegetative cells (132). Subsequent genetic studies identified 3 genes required for MSE^s-dependent vegetative repression: *SUM1*, which encodes a DNA-binding protein that recognizes MSE^s; *HST1*, which encodes a member of the Sir2 family of NAD^+-dependent histone deacetylases (referred to as "sirtuins" below); and *RFM1*, which encodes a protein that links Hst1 to Sum1 (111, 195). Sum1, Hst1, and Rfm1 are not essential for vegetative growth or completion of meiotic development. However, as described below, the Sum1/Rfm1/Hst1 complex regulates meiotic development shortly before commitment is reached.

The *SUM1* gene had previously been identified by a dominant mutation (*SUM1-1*) that suppressed silencing defects of *sir* mutants at the HMR silent mating locus (23, 83, 89, 92, 96). Subsequently it was shown that *SUM1-1*-mediated suppression of *sir2Δ* requires *HST1* and *RFM1* (111, 143, 172). The Sum1-1 protein "spreads" across extended regions of chromatin and is present at HMR, while the wild-type Sum1 protein does not spread and is not present at HMR (101, 143). Hst1 in *Saccharomyces* is similar in sequence to Sir2 (67% identical across 453 residues) and appears to have arisen during the whole-genome duplication event that occurred in the evolutionary history of *Saccharomyces* (54). In *Kluyveromyces lactis* (which diverged from *S. cerevisiae* prior to the duplication), Hst1 does not exist, and the single *SIR2* gene functions in both Sum1-mediated repression of meiotic genes and heterochromatic silencing. Comparative analyses indicate that duplication of an ancestral sirtuin gene followed by mutations that specifically reduced interactions with *SIR* proteins can explain Hst1's specificity for Sum1-regulated genes in *S. cerevisiae* (38, 55). It is possible that the *SUM1-1* substitution uncovers a vestigial function (lost between *K. lactis* and *S. cerevisiae*) involving the ability of Sum1 to spread on chromatin.

MSE variants found in middle meiotic promoters differ in their ability to repress transcription in vegetative cells. Some MSE variants repressed all detectable expression of a heterologous test reporter, other variants repressed expression moderately, and others did not repress at all (195). Moreover, *in vitro* binding experiments showed that the MSE variants that strongly repressed transcription in vegetative cells bound Sum1 tightly, while the variants that did not repress transcription lacked detectable Sum1-binding activity (131). Mutagenesis of MSE^S identified bases required for high-affinity Sum1 and Ndt80 binding. These data, taken in conjunction with computational analyses of MSEs found in Sum1-repressible promoters, led to the Sum1 binding consensus 5′-AG**YGWCACAAAA**D-3′ (where Y is C or T, W is A or T, and D is A, G, or T). Notably, there is overlap between the bases required for Sum1 and Ndt80 binding (underlined), but a dinucleotide upstream of the overlap region (5′-AG-3′), a single base within the overlap, and a base downstream of the overlap uniquely promote Sum1 binding (Fig. 3). Computational algorithms that analyzed genome-wide Sum1 occupancy (chromatin immunoprecipitation [ChIP]) data from vegetative cells, Ndt80-inducible elements, and the transcriptional program of meiosis also grouped MSEs into Sum1-repressible and nonrepressible sets (186). Consistent with these findings, the DNA-binding domain of Ndt80 can competitively displace the DNA-binding domain of
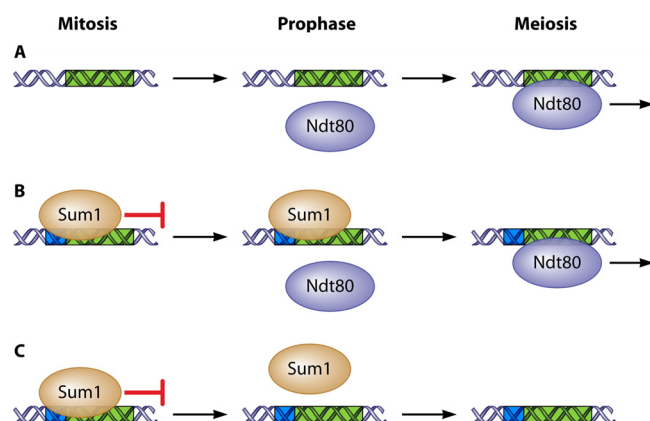
FIG 3 Sum1 and Ndt80 interact with overlapping sites to control meiotic processes. The core MSE (consensus, 5′-YGNCACAAAA-3′) is shown as a green rectangle that can become bound when the Ndt80 protein is produced in meiotic prophase (A). Sum1 recognizes the core MSE, but additional bases (blue rectangle) generate a composite site (consensus, 5′ AG<u>YGWCACAA</u><u>AAD</u>) that can be recognized by both Sum1 and Ndt80. Sum1 can be competitively displaced from DNA by Ndt80 during meiotic prophase (B). However, Sum1 can also be removed in the absence of Ndt80 (C). See the text for details. The consensus sites (where N is any base, Y is C or T, and D is A, G, or T) are taken from reference 131.

Sum1 from MSE^S DNA *in vitro* (131). Moreover, ectopic expression of Ndt80 in vegetative haploid cells can induce the expression of Sum1-repressible genes (25). Since Ndt80 binding and Sum1 binding are mutually exclusive, this indicates that Ndt80 can competitively displace Sum1 from DNA *in vivo*.

In contrast to the case for Ndt80, little is known about the structural basis of Sum1 sequence-specific DNA binding. Although Sum1 and Ndt80 recognize an overlapping DNA element, there is no significant primary sequence similarity between these proteins. The carboxy-terminal half of the 1,023-amino-acid Sum1 protein is sufficient to specifically bind MSE^s DNA (131). However, the amino-terminal half of Sum1 binds A+T-rich DNA, and several "A/T hook" motifs are located in this region of the Sum1 protein (5). A/T hooks collaborate with helix-turn-helix motifs to increase the avidity of protein/DNA interactions in homeodomain proteins (82). The A/T hooks in Sum1 may play a similar role or increase the affinity of Sum1 for a subset of MSEs that are adjacent to A+T-rich DNA.

Genome-wide expression studies demonstrated that about half of the Ndt80-inducible middle promoters are derepressed in *sum1Δ* vegetative cells (131). Sporulation-specific functions such as spore wall morphogenesis are significantly overrepresented in the Sum1-repressible gene set. Genome-wide expression analyses of *hst1Δ* and *rfm1Δ* vegetative cells showed that about half of the Sum1-repressible promoters are also Hst1 repressible (9, 111). The Hst1- and the Rfm1-repressible sets of genes are nearly identical, suggesting that Rfm1's major role is to link Hst1 to Sum1. For all Sum1-repressible genes tested, vegetative expression is greater in a *sum1Δ* mutant than an *hst1Δ* mutant. The vegetative expression of Sum1-repressible genes is greater in a *sir2Δ hst1Δ* double mutant than in the single *hst1Δ* mutant (55). Sir2 can function in place of Hst1 in an *hst1* deletion mutant but not in a catalytically inactive *hst1* mutant. These observations suggest that Sir2 can substitute for Hst1 through an "imposter" mechanism. The vegetative expression of almost all Sum1-repressible genes is substantially higher in a *sum1Δ* mutant than in an *hst1Δ sir2Δ* double mutant or an *hst1* catalytically inactive mutant. These findings indicate that Sum1 represses transcription using a mechanism that requires recruitment of a sirtuin and also by a mechanism that is sirtuin independent. Further work is required to establish how Sum1 represses transcription in the absence of sirtuins.

It is worth noting that Sum1 also regulates genes that are not induced during meiosis. Some of these genes encode enzymes in the *de novo* pathway for NAD$^+$ biosynthesis, and it has been proposed that Hst1 activity at these promoters modulates transcription to maintain NAD$^+$ homeostasis (9). Sum1 also interacts with the a1 homeodomain protein to repress α-specific genes in **a** cells (200). In addition, Sum1 has also been shown to positively regulate a subset of origins of DNA replication (70, 101, 190). Thus, while a major role of Sum1 is to repress middle promoters, Sum1 has additional roles in the cell. The DNA elements at these nonmeiotic promoters appear to contain Sum1 specificity determinants but incomplete MSE core consensus elements. It is possible that protein/protein interactions that depend on other sequence-specific DNA-binding proteins (such as a1) or A/T hook interactions with adjacent A+T-rich DNA play a role in stabilizing Sum1 binding to these sites.

(i) Sum1 is a regulatory brake that controls *NDT80*. The data described above suggest two alternative models for Sum1's role in meiosis. One model posits that Sum1 is a regulated brake that controls middle meiotic promoters in response to dependency, cell cycle, or checkpoint signals. A second model posits that Sum1 functions constitutively to prevent adventitious activation of middle meiosis-specific promoters and that it is simply displaced from DNA by Ndt80 as middle genes are induced. Data in support of both the "regulatory brake" and the "constitutive" models have been reported. For example, *SUM1* was shown to be required for the pachytene checkpoint (94, 127). However, it is unclear whether Sum1 is directly controlled by checkpoint signaling pathways (see below). In addition, genetic studies indicated that Sum1 is negatively regulated by the meiosis-specific CDK-like kinase Ime2 (126), and Ime2 was shown to phosphorylate Sum1 on residue T306 (118). However, a Sum1-T306A mutant (here referred to as the *sum1-i* mutant) completes meiosis with only a modest delay (3).

Insight into the role of Sum1 in meiotic regulation was provided by the observation that *NDT80* is derepressed in a *ume6Δ sum1Δ* double mutant but not in either single mutant (195). This finding raised the possibility that Sum1 can restrict *NDT80* expression but only when Ume6 repression has been relieved (in diploid cells expressing early meiosis-specific genes). Mutational analysis of the *NDT80* promoter showed that it is controlled by 2 URS1s and 2 MSEs (126). One of the MSEs appears to be exclusively Ndt80 inducible, while the second is Ndt80 inducible and Sum1 repressible. Moreover, mutation of the MSEs or deletion of *SUM1* advanced the timing of *NDT80* expression. These studies suggest that Sum1 can prevent pachytene exit by repressing the *NDT80* promoter.

(ii) Two pathways control removal of the Sum1 brake. Sum1-regulated genes are derepressed and Sum1 is removed from MSE DNA in *ndt80Δ* cells that have been transferred to sporulation medium (during prophase) (3). Interestingly, although *sum1-i* cells complete meiosis with nearly wild-type kinetics, Sum1-i protein is inefficiently removed from MSE DNA in *ndt80Δ* cells. In

contrast, Sum1-i is efficiently removed from MSE DNA in *ndt80Δ hst1Δ* cells. These findings suggest that Sum1 can be removed from chromatin by two pathways. The first pathway is *NDT80* independent and involves Ime2 and the downregulation of Hst1, while the second pathway is Ndt80 dependent and involves the competitive displacement of Sum1 (Fig. 3B and C). The existence of an Ndt80-independent pathway for removing Sum1 is consistent with Sum1 being a regulated gatekeeper that controls exit from pachytene by repressing *NDT80*.

(iii) **Control of Sum1 by phosphorylation.** The minimal Cdk1 phosphoacceptor consensus is **S** or **T**-P (the "preferred" consensus is **S/T**-P-X-K/R) (phosphoacceptor residues are in bold), while the consensus for Ime2 is R-P-X-**S/T**-A/V (59, 118). These sites are distinct and nonoverlapping, yet they are often found in closely juxtaposed regions of meiotic proteins (59). Moreover, the regions containing Ime2 and Cdk1 phosphoacceptors often appear to be rapidly evolving based on comparative analyses of genome sequences, and they tend to be in disordered protein segments (60). These observations have led to the proposal that Ime2 and Cdk1 usually coregulate meiotic substrates by bulk electrostatics that influences protein-protein interactions (not through allostery) (59). It has further been proposed that differences in the availability of these sites to phosphatases are important for how Ime2 superimposes meiotic features on the "mitotic default" (Cdk1) pathway. To address the possibility that Ime2 and Cdk1 coregulate the Sum1 brake, a mutant form of the *SUM1* gene that lacks phosphorylatable residues at all 11 minimal Cdk1 consensus sites (*sum1-c*) was generated (155). *sum1-c* cells completed meiosis and formed spores similarly to the wild type. However, when the *sum1-i* (T306A) and the *sum1-c* substitutions were combined, the resulting *sum1-ci* mutant blocked the meiotic program in pachytene with undetectable levels of *NDT80* mRNA or protein. Mutation of the Sum1-responsive MSE in the *NDT80* promoter partially bypassed the *sum1-ci* arrest. These findings suggest that Ime2 and Cdk1 promote progression through pachytene by phosphorylating Sum1 and that the *NDT80* promoter is a critical target of Sum1 regulation. Notably, either *sum1-i* or *sum1-c* blocks the removal of Sum1 repression in *ndt80Δ* cells. These findings indicate that both Ime2 and Cdk1 are required to trigger the removal of Sum1 repression in the *NDT80*-independent pathway. The findings that *sum1-i* or *-c* mutants complete meiosis and form spores suggest that Ndt80 can contribute to Sum1 removal in wild-type cells, likely by a mechanism involving competitive displacement. What is the mechanism of the *sum1-ci* block? *hst1Δ sum1-ci* or *rfm1Δ sum1-ci* cells efficiently complete meiosis and form spores. The suppression of the *sum1-ci* phenotype by *hst1Δ* or *rfm1Δ* raises the possibility that phosphate addition to Sum1 by Cdk1 and Ime2 decreases the activity of Hst1 at Sum1-repressible promoters (perhaps by promoting the dissociation of Rfm1 and/or Hst1).

Further insight into Sum1 regulation comes from studies of the Dbf4-dependent Cdc7 protein kinase (referred to as Cdc7 below). Cdc7 is a key regulator of the cell cycle that is essential for the initiation of DNA synthesis in mitotic and meiotic cells (151). A mutation in the MCM-associated helicase that is targeted by Cdc7 (Bob1/Mcm5) bypasses this requirement (50). Studies of the *bob1Δ cdc7Δ* bypass strain, as well as strains harboring an analog-sensitive allele (*cdc7-as*), have revealed that Cdc7 is required for meiotic recombination. This requirement is because Cdc7 phosphorylates Mer2/Rec107 and thereby promotes interactions required for Spo11-mediated DSB formation (109, 146, 185). Cdc7 also promotes mono-orientation (the attachment of kinetochores to microtubules emanating from a single SPB) by regulating the association of monopolin with kinetochores (98, 109). Thus, Cdc7 is required for meiotic DNA replication and is also required to set up the reductional segregation pattern of MI (106). In addition, *NDT80* is not transcribed and middle genes are not induced in *bob1 cdc7Δ* strains or in *bob1 cdc7-as* diploids treated with the inhibitor (98, 146). Most recently, Lo and coworkers have shown that Sum1 is phosphorylated on multiple Cdk1 sites in mitotic and meiotic cells and on multiple Cdc7-dependent sites specifically in meiosis (97). Moreover, *hst1Δ* and *rfm1Δ* bypass the block to middle gene expression that occurs in inhibitor-treated *cdc7-as* cells. Cdk1 can prime the subsequent phosphorylation of proteins by Cdc7 (24, 108). Taken as a whole, these observations suggest that the phosphorylation of Sum1 by Cdk1 and Ime2 triggers the phosphorylation of Sum1 by Cdc7. The combined activity of these protein kinases may in turn enhance the dissociation of the Rfm1/Hst1 complex from Sum1 and may also promote the dissociation of Sum1 from DNA.

CONTROL OF MIDDLE GENES BY THE PACHYTENE CHECKPOINT

The Pachytene Checkpoint Prevents Ndt80 Activation

As described above, meiotic recombination is initiated by the programmed introduction of DSBs throughout the genome in the leptotene stage of prophase by the meiosis-specific Spo11 endonuclease (80). Mutants that block meiotic recombination after Spo11 has acted arrest in pachytene. The arrest requires the pachytene checkpoint, which delays MI in wild-type cells until recombination intermediates have been repaired, thus protecting against chromosome missegregation events that would lead to aneuploid gametes (56, 137). The pachytene checkpoint shares several components with mitotic checkpoint pathways for DSB repair, including the Rad17/Mec3/Ddc1 PCNA-like clamp, which is specific for unrepaired recombination intermediates (the yeast equivalent of the human 9-1-1 complex), the Rad24 ATPase, which participates in clamp loading on damaged DNA, and the Mec1 phosphatidylinositol (PI) 3-kinase-related protein kinase (the yeast equivalent of human ATR), which regulates a variety of DNA repair processes. The pachytene checkpoint also requires proteins that are not shared with mitotic checkpoint pathways that connect its activity to chromosomal features that are encountered specifically in the meiotic cell (56, 137). One well-studied system that has been used to model the pachytene checkpoint involves deletion of *DMC1*, which encodes a meiosis-specific RecA-like strand exchange factor (a paralog of the Rad51 mitotic recombinase) (11). *dmc1Δ* cells arrest in pachytene with unrepaired DSBs, while the further mutation of checkpoint genes such as *RAD17* or *MEC1* enable cells to progress through meiosis-like divisions and form inviable spores that contain DSBs (100). A second well-studied system that has been used to model the pachytene checkpoint involves the deletion of *ZIP1*, which encodes a structural component of the SC (166, 173, 174, 176).

The mutation of genes encoding checkpoint sensors and signaling enzymes can bypass the pachytene checkpoint response to unrepaired DSBs, as is seen in a *dmc1Δ rad17Δ* or *dmc1Δ mec1-1* background (100). However, progression through pachytene and sporulation can also take place in a *dmc1Δ* background when the

bias for interhomolog recombination is eliminated and repair can therefore occur using sister chromatids of homologous chromosomes. The bias for interhomolog recombination requires a set of proteins that localize to the axial elements of the SC, including Hop1, Red1, and the meiosis-specific Mek1 protein kinase (a paralog of the Rad53 checkpoint kinase) (122–124, 150, 184, 197). Mek1 prevents repair of DSBs using the sister chromatid (the repair template used in mitotic cells) by preventing Rad51 from invading sister chromatids, thus biasing toward interhomolog recombination (58, 124). A *dmc1Δ mek1Δ* strain bypasses the pachytene arrest and forms spores, but it does so through a route that involves the inappropriate (mitosis-like) repair of DSBs using the Rad51 intersister pathway. While the *dmc1Δ mek1Δ* meiotic products do not have DSBs, they were generated in the absence of chiasmata (the sites of reciprocal recombination that link homologs), and the spores are therefore inviable due to massive missegregation at MI. The Mek1 kinase is activated when Hop1 is phosphorylated by Mec1 (20). Thus, Mec1 not only plays a role in the pachytene checkpoint response but also promotes events that are required for the completion of normal meiosis (19). These findings nicely illustrate how core checkpoint functions are interdigitated with meiosis-specific chromosomal processes. It has recently been reported that Mek1 can further modify the checkpoint response by increasing the checkpoint-mediated delay at pachytene (192).

While the pachytene checkpoint senses a variety of different meiotic recombination intermediates (56, 137), the checkpoint signals that are generated in these pathways converge on common targets to regulate exit from pachytene. One critical target of the pachytene checkpoint is cyclin-dependent kinase. The Swe1 kinase, which inhibits Cdk1 by phosphorylating a tyrosine residue near its ATP-binding pocket, becomes hyperphosphorylated and stabilized in response to pachytene checkpoint activation, and *swe1Δ* partially bypasses the pachytene arrest phenotype of a *dmc1Δ* strain (90, 127). A second critical target of the pachytene checkpoint is Ndt80 (2, 26, 53, 176, 187). *dmc1Δ* and *zip1Δ* mutants express *NDT80* but at a reduced level (94, 176). Ndt80 target genes are not induced in these checkpoint-arrested cells, indicating that the Ndt80 protein that is produced is inactive (26, 53). Ndt80 is hyperphosphorylated in wild-type cells and hypophosphorylated in checkpoint-arrested cells (158, 176). These observations suggest that Ndt80 is normally activated by phosphorylation and that these activating phosphomodifications are prevented from taking place when cells undergo checkpoint-mediated arrest. The reduced level of *NDT80* expression in checkpoint-arrested cells is likely a secondary consequence of the inability of the hypophosphorylated form of Ndt80 to participate in the positive autoregulatory loop.

Ndt80 Is Held in the Cytoplasm by the Checkpoint

Ndt80 is found almost exclusively in the nucleus in wild-type meiotic cells, and it is found largely in the cytoplasm in *dmc1Δ* or *zip1Δ* checkpoint-arrested cells. Moreover, expression of a mutant form of Ndt80 (termed Ndt80-bc) which lacks a 57-residue internal stretch of the protein between the DNA-binding domain and the transcriptional activating domain bypasses the checkpoint, and *zip1Δ ndt80-bc* or *dmc1Δ ndt80-bc* strains form spores (mostly inviable). Moreover, Ndt80-bc is nuclear in both checkpoint "on" and "off" states (187). These findings demonstrate that the checkpoint prevents exit from pachytene by regulating the

nuclear localization of the Ndt80 protein. The available data suggest that a cytoplasmic anchor prevents Ndt80 from entering the nucleus when the checkpoint has been activated. Further studies are required to identify the anchor and elucidate how the checkpoint regulates its interaction with Ndt80.

Sum1 Is Required for the Checkpoint Response

While *dmc1Δ* cells arrest at pachytene and do not form spores, a substantial fraction of *dmc1Δ sum1Δ* cells undergo two rounds of chromosome segregation, and many of these cells form spores in the presence of DSBs (94, 127). Thus, *SUM1* is required for the pachytene checkpoint response. In the initial study that connected Sum1 to the pachytene checkpoint, *NDT80* mRNA was expressed in the *sum1Δ dmc1Δ* background yet Ndt80-regulated genes were not detectably induced (94). These observations are consistent with the Ndt80 protein being targeted by the checkpoint. However, in a subsequent study, Ndt80-inducible genes were expressed at nearly wild-type levels in *sum1Δ dmc1Δ* cells (127). In the former study about 35% of the cells bypassed the checkpoint (completed meiosis-like divisions), while in the later study about 70% of the cells bypassed the checkpoint. The difference in Ndt80 activity reported in these studies therefore correlates with extent of checkpoint bypass and may reflect a threshold-like increase in Ndt80 that can occur in *dmc1Δ sum1Δ* cells. Irrespective of this, it is clear that some level of Ndt80 activity is required for the checkpoint bypass phenotype, since *ndt80Δ* cells uniformly block in pachytene (196) and since meiosis-like divisions were undetectable in the *dmc1Δ sum1Δ ndt80Δ* background (127).

Despite the finding that *SUM1* is required for the pachytene checkpoint response, it is still unclear whether Sum1 is a regulated target of the checkpoint. The concentration of Sum1 decreases in meiotic cells prior to pachytene, and increased levels of Sum1 were observed in checkpoint-arrested cells. These observations led to the suggestion that the checkpoint stabilizes Sum1, thereby preventing middle gene induction (94). However, a subsequent study demonstrated that the reduction of Sum1 protein observed in meiotic cells is insufficient to trigger derepression of middle promoters (3). These findings suggest that regulated changes in Sum1 levels are unlikely to play a major role in preventing middle gene expression under conditions of checkpoint-mediated arrest. It has been suggested that the reason *sum1Δ* bypasses the checkpoint is that Ndt80 is produced and activated before a functional response to the checkpoint can take place (126, 127). According to this "kinetic model" Sum1 need not be regulated by the checkpoint. Mec1 phosphorylates Sum1 on S712 in response to DNA damage in mitotic cells (163). However, a nonphosphorylatable *sum1-S712A* mutation fails to cause detectable bypass in a *dmc1Δ* strain (unpublished data). Further work is required to establish the molecular mechanisms that connect Sum1 to the pachytene checkpoint. Taken as a whole, the available data indicate that Ndt80 is the major target that connects persistent recombination intermediates and defects in synapsis to the transcriptional cascade. Therefore, the posttranslational regulation of Ndt80 by phosphorylation can be viewed as a final opportunity for cells to prevent passage through the commitment point (Fig. 4).

REESTABLISHING REPRESSION OF MIDDLE PROMOTERS

Almost all genes that are controlled by transcriptional cascades are expressed transiently. Little is known about how repression of meiosis-specific promoters is reestablished after they have been
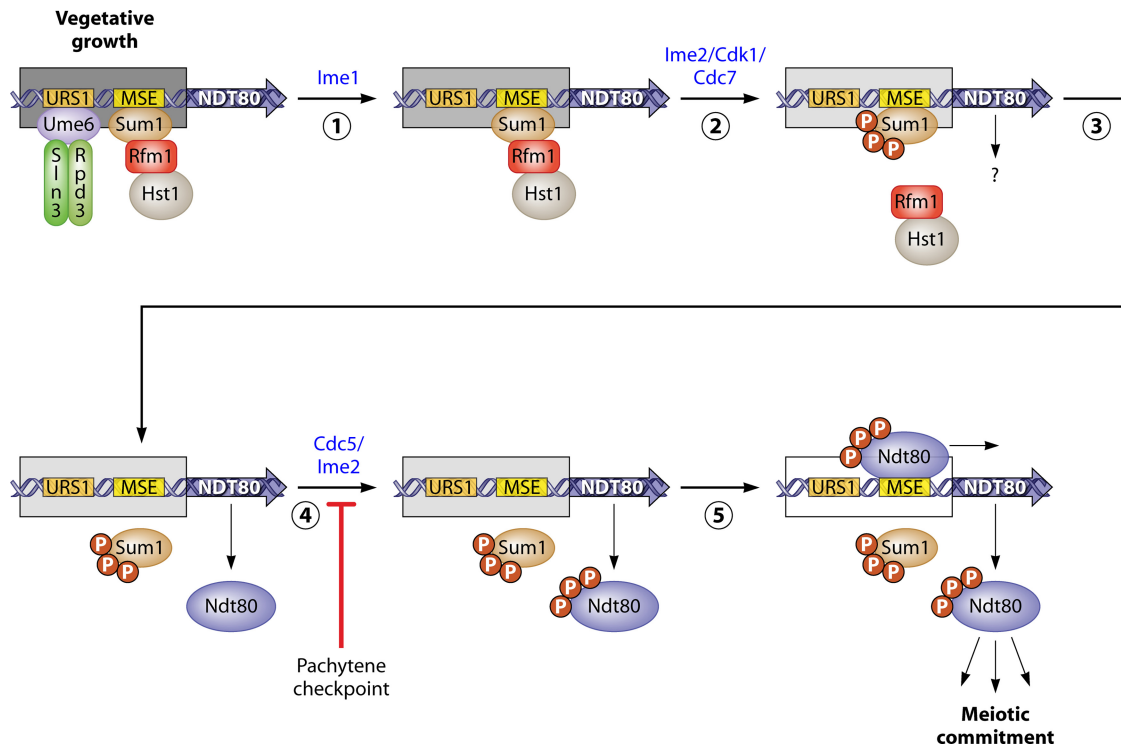
FIG 4 The *NDT80* transcriptional induction pathway. The sequential steps leading to the expression of the *NDT80* promoter are shown. The shaded box represents distinct chromatin structures at the *NDT80* promoter that may be generated as the indicated regulatory transitions take place. Although the *NDT80* promoter contains 2 URS1s and 2 MSEs (one which is Sum1 repressible and Ndt80 inducible and a second that is exclusively Ndt80 inducible), only a single URS1 and the Sum1-repressible/Ndt80-inducible MSE are shown. Ime1 is present at early (URS1-containing) promoters as they are expressed and is therefore predicted to be present after step 1. However, the mechanism that tethers Ime1 to early promoters is unresolved, and for simplicity its presence at URS1 is not diagrammed. See the text for details.

expressed. Genetic studies suggest that the system that reestablishes repression of early meiotic promoters during meiotic development is different from the system that operates in vegetative cells (105). In the case of middle promoters, there is conflicting evidence about how repression is reestablished. In one study, it was reported that *SUM1* is required to reestablish repression of the *NDT80* promoter (84), while another study reported that the *NDT80* promoter is expressed in a transient fashion in a *sum1Δ* mutant, similar to wild-type expression (94). Whether repression of *NDT80* is reestablished in meiotic cells by the Ume6/Rpd3/Sin3 pathway, by the Sum1 pathway, or by both pathways remains to be determined.

CONTROL OF THE *SUM1/NDT80* SWITCH BY NUTRIENTS

As described above, the genome-wide analyses of the RTG response by Freidlander et al. showed that *IME1* and early genes are efficiently repressed in response to rich medium (37). Surprisingly, the expression of most middle genes was also repressed upon addition of rich medium, even in committed cells that continued to sporulate. These findings demonstrate that the completion of the meiotic divisions and spore formation can take place when the expression of most middle genes has been reduced. A small number of middle genes (24 of the 269 genes classified as middle genes in this study) were not repressed in these experiments. Many of these nonrepressed genes appear to be connected to the process of spore wall formation and were expressed as spore wall morphogenesis was taking place. The reduction of middle

gene expression that occurs upon RTG was observed in a *sum1Δ* background. This suggests that most of the decrease in middle gene expression that occurs upon the addition of rich medium is a consequence of Ndt80 downregulation (see below) and/or regulated changes in the stability of middle mRNAs. In addition to the middle genes, a set of genes that were differentially expressed only in committed cells was identified (63 genes that were induced and 50 that were repressed). The expression of these genes was unchanged in unperturbed meiotic cells or when rich medium was added to meiotic cells prior to commitment. These correlations raise the possibility that commitment-specific changes in gene expression take place that can promote the continuation of sporulation when nutrient levels are high.

The addition of glucose to starved vegetative cells elicits dramatic changes in gene expression (74). These changes are mediated largely through the Ras/cAMP/protein kinase A (PKA) pathway (188). In the study by Friedlander et al. (37), glucose/PKA-responsive genes in vegetative cells were compared with those in meiotic cells that had been treated with glucose both before and after commitment. Interestingly, the glucose/PKA transcriptional responses of vegetative cells, precommitment meiotic cells, and spores were similar, while the transcriptional response of postcommitment cells that were completing the meiotic divisions and spore formation was different. These findings suggest that glucose is sensed in postcommitment meiotic cells but that the PKA-dependent transcriptional response is modified to favor completion of the program even when glucose levels

are high. While it seems counterintuitive that postcommitment cells complete sporulation when the expression of most middle genes has been downregulated, most middle gene transcripts are present only briefly in synchronously sporulating cells. These findings indicate that submaximal levels of most middle transcripts are sufficient to promote MI/MII and spore formation (which happen fairly rapidly and in succession), and this may be related to the switch-like properties of the system and irreversibility.

A MODEL FOR MEIOTIC COMMITMENT

As described above, the production of active Ndt80 is the key regulated event that triggers meiotic commitment in wild-type cells. The pathway that produces active Ndt80 can be divided into five steps (Fig. 4). Step 1 requires *IME1* expression, which occurs when diploid cells are exposed to the inducing signal for meiosis (starvation). This step is controlled by the interplay between Ume6, Rpd3/Sin3, and Ime1 at the URS1 elements in the *NDT80* promoter. One likely consequence of these interactions is that Rpd3 activity at *NDT80* is reduced. This is predicted to lead to changes in acetylation that influence the chromatin structure of the *NDT80* promoter (indicated by changes in shading in Fig. 4). These changes require the activity of an unidentified histone acetyltransferase (HAT) that opposes Rpd3. Gcn5 is a HAT that functions to acetylate histone H3 at the *IME2* (early) promoter in meiotic cells (17). However, Rpd3 preferentially deacetylates H4 at the *IME2* promoter. In addition, H3 acetylation at the *IME2* promoter precedes Ime1 binding and does not coincide with transcriptional induction (68). Thus, while Gcn5 functions at early promoters during meiosis, there may be another HAT that opposes the Rpd3-catalyzed reaction. Importantly, the derepression of URS1 sites establishes a state in which the Sum1/Rfm1/Hst1 complex acquires a gatekeeper function at *NDT80* that prevents Ime1-dependent transcriptional activation. Step 2 involves the phosphorylation of Sum1 by Ime2, Cdk1, and Cdc7. Both the Ime2- and Cdk1-dependent phosphomodifications promote the *NDT80*-independent removal of Sum1 from chromatin (3, 155). As discussed above, this requires the induction of early meiotic promoters and persistent starvation signals (*IME2* is expressed as an early meiosis-specific gene and Ime2 is inhibited by nutrients). The G_1- and M phase-promoting cyclins (Cln1 to -3 and Clb1 to -4, respectively) are not present in meiotic prophase. The most likely cyclin subunits that activate Cdk1 for Sum1 phosphorylation are the S-phase-promoting Clb5/6 cyclins, which are present during meiotic prophase (51, 146, 185). Interestingly, *CLB5* and -6 are dramatically induced as middle meiotic genes. It is possible that the induction of *CLB5/6* plays a role in ensuring that Sum1 remains phosphorylated as cells pass through commitment. Whether Clb5 and -6 participate in the Sum1 phosphomodification pathway and how these reactions control the program remain to be determined. Irrespective of the cyclins involved, it seems likely that the Ime2 and/or Cdk1 phosphomodifications promote the phosphorylation of Sum1 by Cdc7 complexed with Dbf4. The findings that *hst1Δ* and *rfm1Δ* bypass the *sum1-ci* pachytene arrest and the requirement of Cdc7 for *NDT80* expression are consistent with Ime2, Cdk1, and Cdc7 promoting the dissociation of the Rfm1/Hst1 complex from Sum1. Direct biochemical studies are required to establish whether this is the case. Changes in chromatin structure that occur as a consequence of Hst1 downregulation require a HAT. The Gcn5 acetyl-

transferase not only functions at early promoters but also works in opposition to Hst1 (47). Gcn5 is therefore an HAT candidate that functions at this step. Hst1 has been shown to remove acetyl groups from K5, K8, and K16 on H4. It has also been shown to deacetylate H3K4ac. H3K4 is also mono-, di-, and trimethylated, and H3K4me is linked to transcription. H3K4 is either methylated or acetylated, and recent data demonstrate that H3K4ac has a positive function in transcription (47). Whether Rpd3 and Hst1 regulate chromatin in a combinatorial fashion and whether Hst1-dependent deacetylation of H3K4 restricts transcriptional activity of *NDT80* remain to be determined. Step 3 involves the dissociation of Sum1 from DNA. Sum1 represses transcription using Hst1-dependent and Hst1-independent mechanisms. It is unclear whether *NDT80* transcription can take place when Hst1 has been removed (prior to step 3, as indicated with a question mark in Fig. 4) or only after Sum1 has been removed from DNA. Step 4 involves the phosphorylation of the Ndt80 protein, which is known to accumulate in a hyperphosphorylated state as middle genes are induced. Persistent recombination intermediates (as seen in a *dmc1Δ* mutant) cause Ndt80 to accumulate in a hypophosphorylated state (176). Ndt80 is positively regulated by Ime2 and Cdc5, which are nuclear enzymes (10, 27, 164). One possibility is that hyperphosphorylation occurs only after Ndt80 has been translocated to the nucleus and that the hypophosphorylated state of Ndt80 observed in checkpoint-arrested cells is caused by Ndt80's retention in the cytoplasm. Thus far, changes in Ndt80 phosphorylation have been inferred from its mobility on electrophoretic gels (10, 158, 176). Further insight into this transition will require identification of the residues in Ndt80 that are phosphorylated and the protein kinases that catalyze the reactions. If cells successfully transition through step 4, they express *NDT80*, and the Ndt80 protein is expected to bind to the Sum1-represssible MSE and further increase the concentration of Ndt80 through the positive autoregulatory loop (step 5).

It appears that there are multiple mechanisms that promote the switch-like behavior of the system. One mechanism involves the ability of Ndt80 to competitively displace Sum1 from DNA. It is possible that derepression of one *NDT80* promoter leads to the competitive displacement of Sum1 from other *NDT80* promoters (there are four copies of the *NDT80* gene in prophase cells). If so, this is expected to contribute to the switch-like properties of the system. A second mechanism that generates switch-like properties is the autoregulatory loop. A third mechanism is that Ndt80-inducible gene products positively regulate Ndt80. For example, Cdc5, the Ndt80-inducible protein kinase that triggers pachytene exit, positively regulates Ndt80 (2, 27, 165). A fourth property of the system that appears to generate switch-like properties is the robust nature of the transcriptional response, which appears to be "overdetermined" and in excess of what is required for cells to transit through meiotic development. Taken together, these mechanisms are expected to contribute substantially to the switch-like increase in *NDT80* transcription and irreversibility of the committed state. Meiotic progression is influenced by *IME1* gene dosage but not *NDT80* gene dosage (48). These observations suggest that a switch from a graded to a threshold mode of transcriptional regulation occurs at the pachytene exit transition. Thus, once cells have begun to induce Ndt80, they complete the program even when Ndt80-dependent gene induction is downregulated by nutrients (37).

AGING AND *NDT80*

Somatic cells age and have a finite capacity to divide. However, in animals, age-related phenotypes are not inherited by progeny. Yeast cells divide asymmetrically, and mother cells give rise to smaller daughter cells. The replicative life span (RLS) of yeast is defined as the number of times a cell can divide, and this can be determined by counting "bud scars" on the surface of a mother cell or by following the ability of a mother cell to bud over its lifetime. It has been known for over 50 years that a mother cell can divide only about 20 times (although RLS varies with strain background) (8, 119). Recently, Unal and coworkers demonstrated that RLS is reset during sporulation in yeast (177, 178). Thus, spores formed by old cells have the same RLS as spores formed by young cells. Moreover, age-associated phenotypes, including increased protein aggregation, abundance of extrachromosomal ribosomal DNA circles (ERCs), and aberrant nucleolar morphology, are reversed following sporulation. The apparent reversal in aging is independent of the meiotic divisions and occurs around the time that spore morphogenesis is occurring. Most remarkably, the transient ectopic expression of Ndt80 in mitotic cells is sufficient to extend the RLS of mitotically growing old cells and even extends the RLS of young cells. The aberrant nucleolar morphology seen in aged cells was reversed following ectopic Ndt80 expression, while the increases in protein aggregation and ERCs were not. One possibility is that the induction of one or more Ndt80-inducible genes is sufficient to promote a youthful RLS. Chromatin structural changes that accompany the Sum1/Ndt80 transition may also be involved. A moderate increase in *SIR2* gene dosage can extend RLS, and calorie restriction has been reported to extend life span in a Sir2-dependent fashion (76, 93). Hst1 and Sir2 can share functional properties under certain conditions. In addition, Hst1 regulates the transcription of NAD^+ biosynthesis genes and therefore provides a link to calorie restriction (9). One consequence of ectopic Ndt80 expression is the displacement of Sum1 (and associated Hst1) from DNA. While these findings suggest that transient displacement of Hst1 from chromatin may play a role in promoting a youthful RLS, displacement of Sum1/Hst1 from DNA does not appear to be sufficient, since Sum1 is removed from DNA in *ndt80Δ* cells and RTG of *ndt80Δ* cells is insufficient to extend RLS. Thus, an Ndt80-inducible gene (or genes) may be required, although an additional requirement for displacing Hst1 from DNA cannot be ruled out. Functional analyses of Ndt80-inducible genes may therefore lead to new insights into the aging process and how it can be reversed.

EVOLUTIONARY CONSERVATION AND THE *SUM1/NDT80* SWITCH

Middle meiotic promoters provide a glimpse into how transcriptional regulatory circuits change over evolutionary time. Proteins similar to Cdk1, Ime2, and Hst1 are encoded by the genomes of a broad range of evolutionarily diverse organisms from yeast to humans. Ndt80 and mammalian p53 use the same domain structure to recognize their cognate DNA elements (177). In mammals a p53 ortholog (p63) is expressed in female germ cells during dictyate arrest (the stage of MI prophase immediately following pachytene at which developing eggs are held until sexual maturity). It has been proposed that p63 is the primordial member of the p53 family that functions in genomic stability of the germ line and that p53 (and p73) evolved for tumor suppression in vertebrate somatic cells (171). It will be of interest to learn whether

studies of Ndt80 in yeast can inform studies of p63 in mammals. *NDT80* is present in all fungi examined (152), yet it appears to have duplicated, evolved additional functions, and lost its connection to meiosis. For example, in *Aspergillus nidulans*, the *NDT80* homolog (*xprG*) is not required for meiosis but is required for nutritional responses (79). In *Neurospora crassa*, several meiotic genes, including *IME1*, *UME6*, and *SUM1*, are absent, yet there are three *NDT80* family members. Two of the *NDT80* members (FSD-1 and VIB-1) control female sexual development and spore maturation, yet none are required for meiosis and even the mutant lacking all three members completes meiosis (67, 194). In *Candida albicans* (which does not undergo meiosis), the Ndt80 homolog (CaNdt80) appears to bind to DNA elements that conform to the *S. cerevisiae* MSE, yet the CaNdt80/MSE interactions control genes that regulate sterol biosynthesis, hyphal growth, and cell separation (152, 153).

Similar to Ndt80, Ime2 has taken on diverse roles in different fungal species. While the predominant role of Ime2 in *Saccharomyces* is to regulate meiosis, it also regulates pseudohyphal dimorphism in some *Saccharomyces* strains (169). In the evolutionarily distant fission yeast *Schizosaccharomyces pombe*, there are two Ime2 homologs that redundantly control spore formation (but not meiosis) (1). In *Aspergillus nidulans* Ime2 controls sexual development in response to red light (13). In *Neurospora*, ime-2 appears to control sexual development by regulating one of the *NDT80* homologs (VIB-1) (67). In *Cryptococcus neoformans* Ime2 has been connected to mating (95), and in *Ustilago maydis* Ime2 controls pathogenesis (44). This remarkable diversity of Ime2 in fungi has recently been well reviewed (71). In mammals, two Ime2-like protein kinases named MAK and MRK/ICK exist. MAK is expressed to high levels in meiotic cells in the testis, yet it is also expressed in the retina and is induced by androgen in prostate cancer cells (12, 110, 193). MAK and ICK share substantial similarity with Ime2 in their mechanism of activation (40, 41, 147–149, 183). Moreover, both MAK and Ime2 appear to regulate substrates that are also regulated by CDKs, and the phosphoacceptor consensus sequences of Mrk (R-P-X-**S/T**-P) and Ime2 (R-P-X-**S/T**-A/V) are almost identical (40, 59, 118). Despite these mechanistic similarities, MRK is not required for meiosis ($MRK^{-/-}$ transgenic animals are fertile) (156). Recently, ICK, which is found in intestinal crypts, has been shown to be expressed at high levels in cancer cells and to regulate intestinal cell differentiation and cell cycle progression (39). Whether MRK or ICK controls commitment transitions in somatic cells and the extent to which Ime2 regulation resembles MRK and ICK regulation remain to be determined.

REGULATORY LOOPS AND IRREVERSIBLE SWITCHES

In this review I have discussed a transcriptional switch in *Saccharomyces* that controls commitment to meiotic development. This switch controls what is arguably the most important life cycle decision for yeast and as such is likely to have been shaped by substantial evolutionary pressures. There has been much debate and speculation concerning the involvement of chromatin modification in maintaining developmental decisions. Although a sirtuin (Hst1) is involved in this regulatory transition, in the meiotic commitment control model described here, hard-wired positive autoregulatory loops (similar to those first described for phage lambda) appear to be sufficient to explain the switch-like and irreversible properties of this system. The

genetic switch for lysis/lysogeny in phage lambda shares certain features with the meiotic commitment switch described here. These shared features include transcriptional activators and repressors (cI and Cro) that can compete for occupancy *in vitro* and positive autoregulatory loops (134). In eukaryotic systems there are numerous examples of transcriptional repressors and activators that bind to overlapping DNA elements, and many of the promoters controlling these proteins are themselves controlled by their own products. Prominent examples include the E2F proteins and the Gli transcription factors (which control commitment to S phase and developmental decisions, respectively) (22, 69, 142). Challenges for the future include establishing the extent to which repressor/activator pairs and shared DNA sites control commitment processes in animals, identifying the molecular reactions that control these transitions, establishing how chromatin modifications regulate these processes, and developing approaches to short-circuit these switches and revert differentiation programs.

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